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A STUDY OF PAROTID SALIVATION IN THE HORSE

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SUMMARY

1. Saliva flowed from the horse's parotid duct only during mastication.
2. The surface-active local anaesthetic administered by mouth inhibited salivary secretion.
3. Salivary secretion was stimulated by pilocarpine and inhibited by atropine.
4. The volume and composition of saliva secreted in 24 hr from one parotid duct was determined.
5. The concentration of sodium, potassium, calcium, chloride and bicarbonate depended upon the rate of flow. The highest concentrations of these electrolytes were observed during periods of high flow rates.
6. Horse parotid saliva contained a high concentration of calcium.
7. In the absence of a dietary supplement of sodium bicarbonate, the sodium concentration of the saliva fell after about 21 days. There was an associated increase in the potassium concentration. The addition of a sodium supplement restored the sodium concentration of the saliva within 24 hr.

INTRODUCTION

The earliest study of the factors influencing the flow of the horse's parotid saliva was made by Colin (1886) who cannulated the parotid duct and, by means of a silver cannula connected by rubber tubing to a small glass phial secured to a halter, collected the saliva secreted during fixed intervals of time. He was able to show that saliva only flowed when food was being masticated. However, his studies of the chemical composition of the saliva were not very informative, because of the analytical methods available.

The most important study of parotid salivation in the horse subsequent to the work of Colin was that of Scheunert & Trautman (1921). These workers transplanted the papilla of the parotid duct to the side of the face and observed that the saliva was secreted in spurts, the first spurt occurring after 10–20 movements of the jaw. They were able to confirm

Colin's observation that the stimulus to secretion was the mechanical effect of chewing. They showed also that, the more saliva secreted during a fixed period, the higher the ash and chloride content of that saliva.

The purpose of the present investigation was to extend these observations by studying some of the factors involved in the production of parotid saliva and changes in its composition.

METHODS

Observations were made on three ponies, all of which were geldings. In earlier experiments, the parotid duct was cannulated in a similar fashion to that described by Colin (1886). However, despite the use of a number of different materials for the actual cannulae, the preparations only remained viable for periods of 2–3 months, after which time the cannula became detached and the fistulae eventually closed. Most experiments were performed on two ponies prepared by the Pavlov method of exteriorizing the parotid papilla. These animals have been maintained in good condition for over 2 years.

The outflow from the parotid duct over short periods of time was recorded kymographically by means of a photo-electric drop recorder and an integrating recorder. Movements of the jaw were recorded by suspending a sausage-shaped balloon under the lower jaw by means of a strap over the nasal bones and connecting the balloon to a strong rubber tambour recording on a smoked drum. Determinations of the rate of flow and electrolyte composition were made by passing a short polythene cannula of 2 mm diameter into the duct and collecting serial samples into measuring cylinders over fixed intervals of time. Collections of the saliva secreted during a 24 hr period were made by introducing a polythene cannula into the duct, securing this to the face by means of a fine elastic band and collecting the saliva in a rubber bag of 5 l. capacity. The rubber bag was enclosed in a canvas sack suspended by a strap round the pony's neck. With one animal, it was necessary to enclose the whole of this collecting apparatus by means of a long canvas hood covering the pony's head and neck. Urine was collected by means of a rubber funnel strapped under the belly enclosing the penis. The urine flowed from the funnel through a polythene tube of 4 cm internal diameter into a polythene container.

Determinations of the concentration of sodium and potassium in saliva were made by means of an Eel flame photometer. The calcium concentration was determined by the method of Trinder (1960), the chloride concentration by the Eel chloride meter, bicarbonate by the method of Conway (1962) and urea by the urease method described by Wootton (1964). The same methods were used in the determination of concentration of the various electrolytes in blood. Samples required for the determination of bicarbonate and pH were collected under liquid paraffin. pH was measured with a Marconi pH meter and glass electrode.

The ponies were maintained on a diet of hay and water *ad libitum*, supplemented by about 1 lb (454 g) bran and crushed oats/day. Unless the effect of salivary depletion was being observed the bran and crushed oats were mixed with 20 g sodium bicarbonate/day to replace the loss of sodium.

RESULTS

The effect of mastication and drugs on salivation. The effect of mastication on the flow of parotid saliva is shown in Fig. 1. The flow of saliva began shortly after the onset of mastication and only stopped when mastication was discontinued. The sight of food to a pony which was not feeding produced signs of impatience but no salivation. When local anaesthetics readily absorbed from mucous membranes, such as cinchocaine and

amethocaine, were incorporated in the food, salivation stopped shortly after the horse had eaten the medicated food despite the fact that masticatory movements and ingestion of the food continued. Lidocaine, when administered in this fashion, did not completely inhibit salivation, but reduced the flow. Procaine, a local anaesthetic poorly absorbed from mucosae, was without effect. The flow of parotid saliva in the absence of mastication could be stimulated by the administration of pilocarpine, but

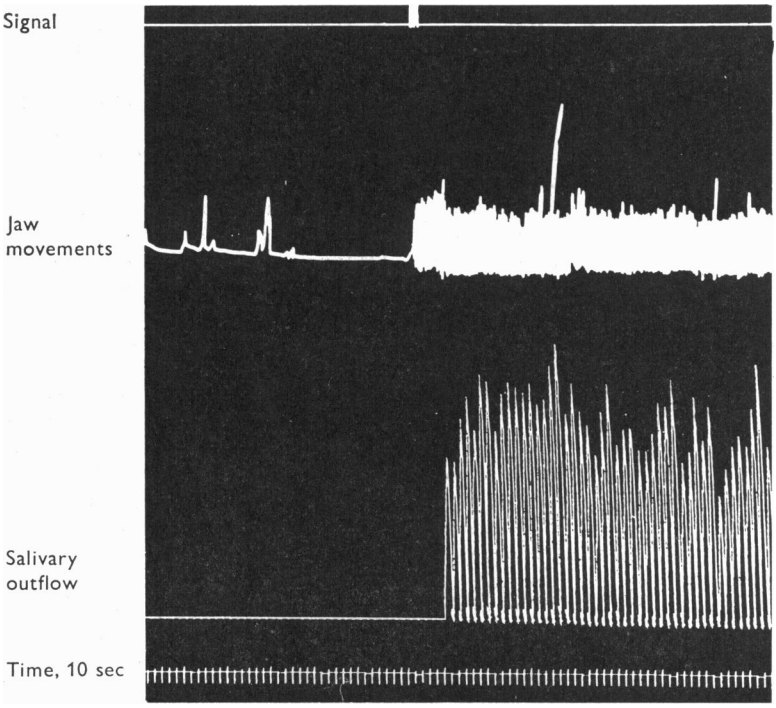

Fig. 1. The induction of parotid secretion by mastication. Hay fed at signal.

even the administration of repeated doses of pilocarpine did not produce a rate of salivary flow as great as that usually obtained during normal feeding. Salivary secretion, however initiated, was completely inhibited by atropine and could not be stimulated by mastication. The effect of orally administered amethocaine on salivary flow is shown in Fig. 2 and of subcutaneously injected pilocarpine and atropine in Fig. 3.

The composition of saliva

The effect of rate of flow. When random samples of saliva were taken and the concentration of the various electrolytes determined, the variation in composition of the various samples was very marked. Experiments were

undertaken to determine the possible cause of this variation and the first possibility investigated was the effect of the rate of flow. It was found that the concentration of sodium, potassium, calcium, chloride and bicarbonate had a linear relation with the rate of flow; the faster the flow, the higher

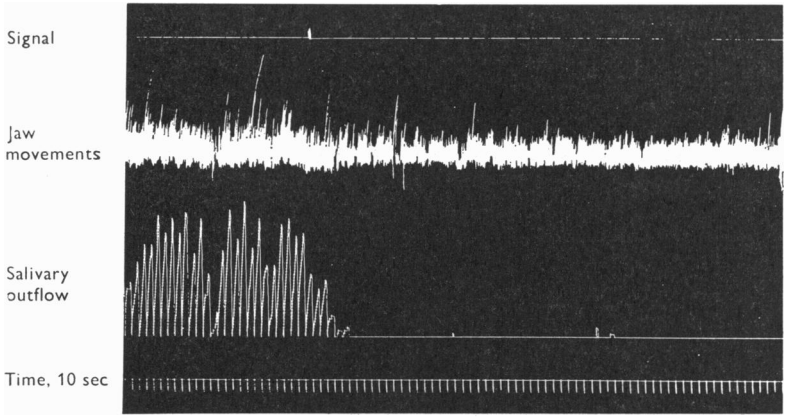

Fig. 2. The effect of parotid secretion of 250 mg amethocaine in the feed. Amethocaine added at the signal.

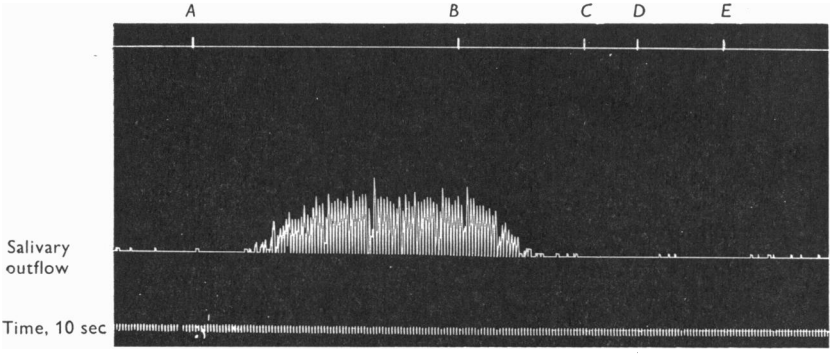

Fig. 3. The effect of 0·3 mg/kg pilocarpine nitrate injected subcutaneously at *A* and 0·2 mg/kg atropine sulphate injected at *B* on salivary secretion. Hay fed at *C*, oats at *D* and sugar at *E*.

the concentration of electrolyte. A typical experiment relating the concentration of sodium to the rate of flow is illustrated in Fig. 4. The calculated coefficients of correlation showing the linear relation between rate of flow and concentration of these various electrolytes are shown in Table 1. There did not appear to be any correlation between rate of flow and concentration of urea.

It was clear from the dependence on the rate of flow of the concentration of the various electrolytes in the saliva that random samples of saliva were

of little use for analysis. Therefore, collections were made of the saliva secreted over a 24 hr period and these collections were analysed for the various electrolytes and urea. The results of the analyses are shown in Table 2. This Table also shows the mean value of saliva secreted over a 24 hr period and the pH of the saliva.

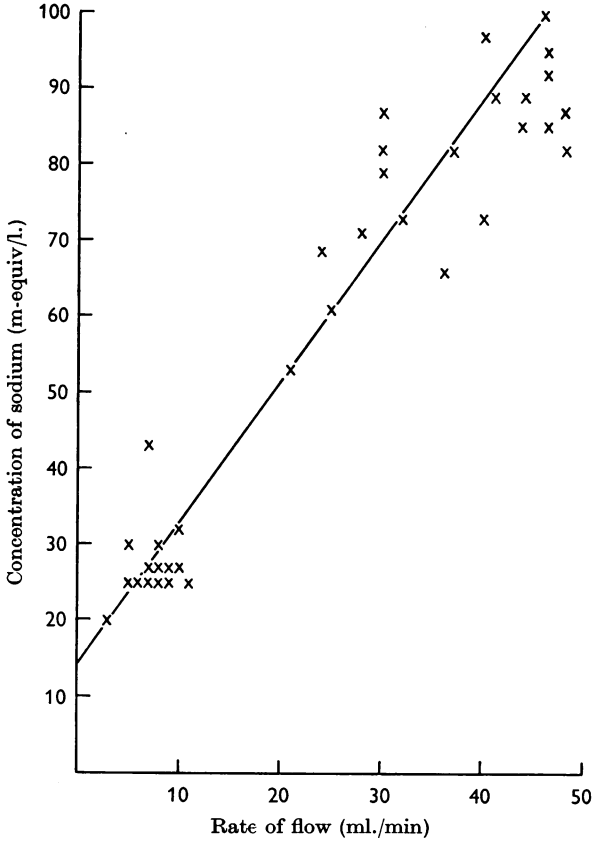

Fig. 4. The relation between the concentration of sodium and the rate of salivary secretion.

TABLE 1. Correlation between rate of flow and concentration of the various salivary electrolytes

Electrolyte	Coefficient of correlation (r)	Number of observations
Sodium	0·56	39
Potassium	0·88	41
Calcium	0·71	27
Chloride	0·96	38
Bicarbonate	0·70	16

The effect of continuous salivary loss upon the electrolytes in saliva. With the fistulated ponies on an unsupplemented diet of hay with approximately 1 lb (454 g) of mixed crushed oats and bran/day, the continual loss of saliva caused a fall in the concentration of sodium in the 24 hr sample of saliva. In consequence, after about 15 days of continual loss, the amount of sodium lost in the saliva was very much less than when the pony received the sodium bicarbonate supplement. The effects of a low sodium diet together with the continual loss of saliva had a somewhat quicker effect in reducing the sodium excreted in the urine and the sodium content of faeces.

TABLE 2. Mean composition of 24 hr collection of parotid saliva

	Concentration (m-equiv/l.) ± S.D.	Animal	Number of observations
Sodium	56·0 ± 7·0	Pony II	17
	54·0 ± 14·7	III	16
Potassium	15·0 ± 2·0	Pony II	17
	14·0 ± 3·7	III	16
Calcium	13·6 ± 1·7	Pony II	17
	12·4 ± 0·8	III	16
Magnesium	3·4 ± 0·04	Pony II	8
	3·3 ± 0·05	III	8
Chloride	50·0 ± 13·0	Pony II	17
	48·0 ± 12·7	III	16
Bicarbonate	52·0 ± 5·6	Pony II	17
	44·0 ± 7·8	III	16
Phosphate	0·25 ± 0·002	Pony II	8
	0·28 ± 0·008	III	8
Volume secreted	6·4 ± 1·4 L	Pony II	17
	4·9 ± 2·0 L	III	15
pH	7·49 ± 0·18	Pony II	20
	7·49 ± 0·09	III	20
Urea	(mg/100 ml.)		
	14·4 ± 1·13	Pony II	8
	14·1 ± 2·31	III	8

When sodium bicarbonate was added to the diet, there was a rapid increase in the concentration of sodium in the saliva and the excretion of sodium in urine and faeces. The results from a typical experiment are shown in Fig. 5. The effect of the continual loss of saliva upon the concentration of potassium in the daily secretion was to produce an increase in salivary potassium concentration during the time at which the sodium concentration was low. The continuous loss of sodium by the animal through salivary secretion did not influence the daily volume of saliva secreted; this is shown in Fig. 5. The effect of the bicarbonate supplement on the faecal excretion of sodium and potassium is shown in Fig. 6.

Witholding the sodium bicarbonate supplement had an appreciable effect upon the amount of urinary sodium and the volume of urine excreted. In the absence of the supplement not only was much less sodium excreted,

but a lower volume of urine was secreted. Although horse parotid saliva had a high calcium concentration, the continual loss of saliva did not appear to affect the concentration of this electrolyte.

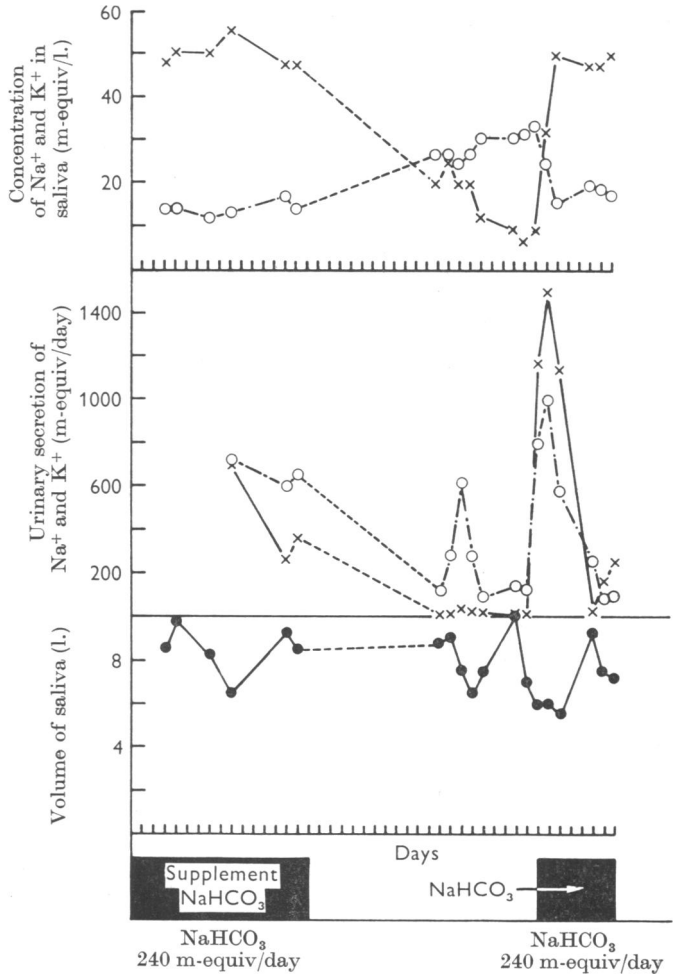

Fig. 5. The effect of witholding the food supplement of sodium bicarbonate on the concentration in saliva of Na^+ and K^+, the urinary secretion of Na^+ and K^+ and the volume of saliva secreted in 24 hr. O—O Potassium, × — × sodium.

DISCUSSION

The observations made by earlier workers on the factors influencing the flow of saliva from the horse's parotid duct have been confirmed. As found by both Colin (1886) and Scheunert & Trautmann (1921) the main stimulus to the secretion of saliva from the parotid duct in the horse was

the mechanical effect of chewing. Since the effect of chewing was abolished when local anaesthetics capable of anaesthetizing mucous membranes were incorporated with the food and was unaffected by local anaesthetics with poor anaesthetizing actions on mucosae, it seemed that the chewing stimulated sensory nerve endings in the buccal mucosa. The responses of the horse's parotid gland to pilocarpine and to atropine were as would have been expected from the effects of these drugs in other species.

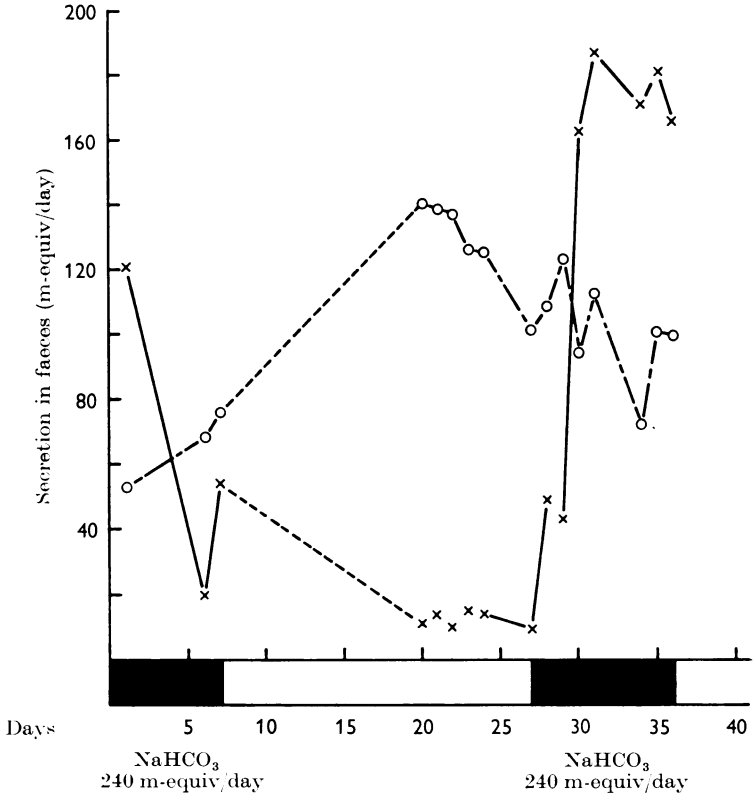

Fig. 6. The effect of witholding the sodium bicarbonate food supplement on the daily excretion in the faeces of sodium and potassium. ○—○ Potassium, × — × sodium.

Although Scheunert & Trautmann (1921) found that the ash and chloride content of saliva increased with the rate of flow of saliva, the analytical methods were not suited to study the composition of small samples and the correlation between concentration and flow. Since neither Scheunert & Trautmann nor Colin were able to collect saliva over 24 hr, they were unable to study the effect of continual loss on the composition. Although the method described here for the collection of a 24 hr sample

of saliva was usually satisfactory, on several occasions, the pony was able to remove the cannula from the parotid duct, usually by rubbing the side of the face along the side of the stall. This made it difficult to be sure of obtaining 24 hr samples over a succession of days. There was, however, little difficulty in obtaining the saliva secreted during the time the pony was eating a meal and this enabled observations to be made on the relation between the composition of the saliva and the rate of flow.

From the results shown in Table 1, the lowest value of r is that for sodium 0·56. Fisher & Yates (1949) give $P > 0{\cdot}001$ for forty observations when $r = 0{\cdot}4896$ and $P > 0{\cdot}01$ when $N = 10$ and $r = 0{\cdot}7079$. Hence it is highly likely that, over the range studied, the concentration of sodium, calcium, chloride and bicarbonate in the saliva is directly related to the rate of flow. The linear relation found between the concentration of sodium, calcium and chloride with the rate of flow is in agreement with results obtained from other species (Burgen & Emmelin, 1961). However, the observations on the pony showed a similar linear relation between the concentration of potassium and the rate of flow and no evidence was found of the U-shaped curve relating potassium concentration with flow found by Burgen (1956).

When the parotid saliva was lost by the pony over a period greater than 15 days and the animal was on a low sodium intake, the loss of sodium in the saliva decreased. There was a reciprocal increase in the concentration of potassium in saliva to correspond with the fall in sodium similar to that found by Denton (1956) in sheep, and an associated decrease in the urinary excretion of sodium which preceded the fall in salivary sodium by several days. The changes in sodium content of saliva and urine in sheep with parotid fistulae and on a low sodium intake (Denton, 1957) took place more rapidly than similar changes in the horse saliva and urine. A probable reason for this might lie in the fact that the concentration of sodium is much greater in sheep's parotid saliva and hence the sheep becomes more rapidly depleted. The addition of sodium bicarbonate to the daily diet of the fistulated pony caused a rapid increase in the sodium concentration of the saliva and a fall in potassium concentration. It would be of interest to see whether the similarity in the changes in the Na:K ratio produced in both the horse and sheep saliva by sodium depletion extended to the control exercised by the adrenal cortex on the Na:K ratio in sheep (Denton, Goding & Wright, 1959). However, the intermittent nature of parotid salivary secretion in the horse would make it unsuitable for assays such as those carried out on the sheep (Denton *et al.* 1959). Sodium depletion caused the urinary sodium to fall quickly and profoundly and also had a marked effect on the faecal excretion of sodium. Denton (1957) observed a similar fall in faecal sodium in the sodium deplete sheep.

It has been shown that a lactic acid producing fermentation takes place in the horse's stomach (Alexander & Davies, 1963) and this would be facilitated by the addition of a bicarbonate buffer. Hence, a possible function for the large volumes of saliva secreted by the pony might be to buffer the fermentation taking place in the stomach. However, the buffering properties of horse parotid saliva fall far short of those of the sheep, which not only contains twice as much bicarbonate, but also contains a substantial proportion of phosphate (McDougal, 1948).

It has been pointed out by Denton (1957) that in composition, the sheep's saliva resembles the dog's pancreatic juice and it is interesting to note that, despite the similarity in natural diet of the sheep and horse, the saliva of the latter species resembles that of the dog, cat and man (Burgen & Emmelin, 1961). However, the high calcium content of horse saliva seemed noteworthy but difficult to explain and the phosphate concentration of horse parotid saliva was lower than in most other species. The total phosphate concentration of saliva in most species is about twice that of the plasma (Burgen & Emmelin, 1961), whereas in the horse, the plasma level of phosphate was nearly 6 times that of the saliva (Alexander, 1962). The level of urea in horse saliva resembled that in sheep (Macdonald, 1948), in which species it contributes to the animal's protein being synthesized by the rumen organisms into bacterial protein which is then digested by the sheep. It is possible that the salivary urea might be used by the organisms involved in the gastric fermentation in the horse as a source of non-protein nitrogen to form bacterial protein.

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